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AN ULTRASTRUCTURAL ANALYSIS OF CYTOPLASMIC LOCALIZATION IN *CHAETOPTERUS PERGAMENTACEUS*

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Abstract

During early development of *Chaetopterus pergamentaceus*, cytoplasmic components visible by electron microscopy became segregated. The hyaloplasm, or spongy layer, composed of granular bodies surrounded by a matrix of endoplasmic reticulum and microtubules was segregated towards (1) the upper pole of the unfertilized egg, (2) the interior of the cleaving egg, (3) the superficial regions of the 16-cell stage, and (4) the ectoderm of the trochophore larva. The ectoplasm, characterized by cortical granules, surrounded the unfertilized and cleaving egg endoplasm. It was restricted to the apical end of cells at the 16-cell stage, and to the ectoderm of the trochophore larva. The endoplasm, characterized by yolk and lipid, (1) was found throughout the unfertilized egg except at the upper pole, (2) was restricted to a ring just below the cortex of the cleaving egg, (3) moved to the basal ends of blastomeres by the 16-cell stage, and (4) was limited to the endoderm of the trochophore. Granular bodies were found throughout the embryo. Mitochondria, found throughout the cytoplasm by first cleavage, were absent from the hyaloplasm of the unfertilized egg. The cytoplasm of the polar lobe was similar to that of the surrounding endoplasm. The localization of morphogenetic determinants was thus accompanied by cytoplasmic organelle localization in *Chaetopterus*. The results are interpreted in terms of localization mechanisms involving the embryo cortex and ground substances.

Introduction

Cleavage subdivides and segregates substances in the egg. This process is called localization, and localization patterns specify the differentiation of cells and tissues (see Wilson, 1928; Davidson, 1976; Whittaker, 1979; Freeman, 1979, for reviews). Localization of morphogenetic determinants may be reflected in the organization of visible cytoplasmic structure. The most clear-cut example of this occurs in *Styela* and related ascidians. In these animals, plasms visible by light (Conklin, 1905) or electron microscopy (Berg and Humphreys, 1960) are segregated into specific cell lineages.

The localization of morphogenetic determinants has been shown experimentally in spirallian eggs (e.g., Wilson, 1904; Novikoff, 1938, Clement, 1952), but attempts to associate particular cell lineages with cellular structure in these eggs usually have been fruitless. Most such attempts have centered on the structure of the polar lobe (see Dohmen and Verdonk, 1979, for review). The egg of the mollusc, *Bithynia*, has a very small polar lobe, containing a structure called the vegetal body, which is associated with the development of lobe-dependent structures (Dohmen and Verdonk, 1979). On the other hand, no structures are restricted exclusively to the

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Abbreviations: MFSW, millipore-filtered seawater.

polar lobes of *Mytilus* (Reverberi and Mancuso, 1961; Humphreys, 1964), *Ilyanassa* (Crowell, 1964), or *Dentalium* (Reverberi, 1970; Dohmen and Verdonk, 1979). This is not surprising, since the larger cytoplasmic organelles can be displaced by centrifugation without affecting subsequent polar lobe formation and normal development (Lillie, 1909; Wilson, 1929, 1930; Whitaker and Morgan, 1930; Morgan, 1933, 1935; Clement, 1968). These results led the early investigators to propose that morphogenetic determinants are localized in the cytoplasmic "ground substances" (Lillie, 1906, 1909) or in the cortex (Morgan, 1933, 1935). These hypotheses have not been tested using the resolution of the electron microscope.

Lillie (1906) showed that cytoplasmic granules, detectable by differential staining, are localized in *Chaetopterus* eggs and embryos. In addition, if the eggs are briefly treated with excess KCl, they redistribute their cytoplasmic components and form cilia, a process called "differentiation without cleavage" (Lillie, 1902; Brachet, 1937). Because of these observations, the embryo of *Chaetopterus* should provide an excellent system for analyzing cytoplasmic organization by electron microscopy. This paper presents morphological evidence that cytoplasmic components, seen and identified by electron microscopy, are localized to different regions of the egg, and that this localization is maintained as the embryo cleaves and cells differentiate. It also presents evidence regarding the organization of both the cortex and the ground substance.

Materials and Methods

Embryonic material

Mature specimens of *C. pergamentaceus* were obtained from the Marine Resources Division, Marine Biological Laboratory, Wood Hole, MA, and removed from their tubes. Sexes were separated and groups of individuals were kept in large fingerbowls with running sea water. Worms remained healthy under these conditions for at least 2 weeks. Females were used only after they had been kept separate from males for at least 2 days. Gametes were obtained and handled by standard procedures (Costello and Henley, 1971). Eggs were obtained by cutting off parapodia, teasing the eggs out, and filtering them through four layers of cheesecloth. Eggs were then washed at least three times with Millipore-filtered sea water (MFSW). Sperm, which oozed from cut parapodia into MFSW, were used to fertilize eggs as soon as the sperm became motile. In all experiments reported, >95% of inseminated eggs cleaved normally and developed into trochophore larvae. Embryos were cultured at 23°C in MFSW in Syracuse dishes and their development was monitored by phase-contrast microscopy.

Cytological techniques

Embryos were fixed either in 4% paraformaldehyde; 5% glutaraldehyde in MFSW (Karnovsky, 1965); or 0.75% glutaraldehyde, 5% formalin, 3% NaCl, and 4.5% sucrose in 0.1 *M* phosphate buffer (Fahrenbach, 1969). Morphology was similar with both fixatives. Aldehyde fixation was for 10–20 min at 23°C. Specimens were washed with MFSW 2 h to overnight, postfixed 30 min in 1% OsO_4 in MFSW, dehydrated rapidly through ethanol, and embedded in Spurr's (1969) low viscosity medium. Thick (1.0 μm) sections were cut using glass knives on a Porter-Blum MT2-B ultramicrotome and stained with toluidine blue. Thin sections were cut

using a diamond knife and stained with uranyl acetate and lead citrate (Reynolds, 1963).

Results

Unfertilized egg

C. pergamentaceus eggs are shed as primary oocytes and spontaneously undergo germinal vesicle breakdown. Unfertilized eggs examined in this study were at first meiotic metaphase. The surface of the egg possessed microvilli, which penetrated a 1 μm thick fibrillar vitelline layer (Fig. 1). A layer of cortical granules lay below the plasma membrane, but these were absent in the region of the meiotic spindle (Fig. 2). The spindle region had a spongy appearance due to numerous non-membranous vesicles, which often contained granular material (granular bodies). The spongy region also contained networks of microtubules and endoplasmic reticulum (Fig. 2) and was completely devoid of yolk, mitochondria, and lipid, except at its periphery. Granular bodies appeared to be arranged in rays by the spindle (Fig. 2, inset). At high magnification, they were composed of particles, 18–20 nm in diameter, and often were found in chains (Fig. 4). The rest of the cytoplasm contained a variety of organelles, apparently distributed haphazardly (Figs. 1, 3). Yolk platelets were interspersed with lipid, mitochondria, smooth endoplasmic reticulum, and granular bodies structurally similar to those in the spindle (Fig. 3). At high magnification, the yolk had a paracrystalline structure (Fig. 5).

Cleaving egg

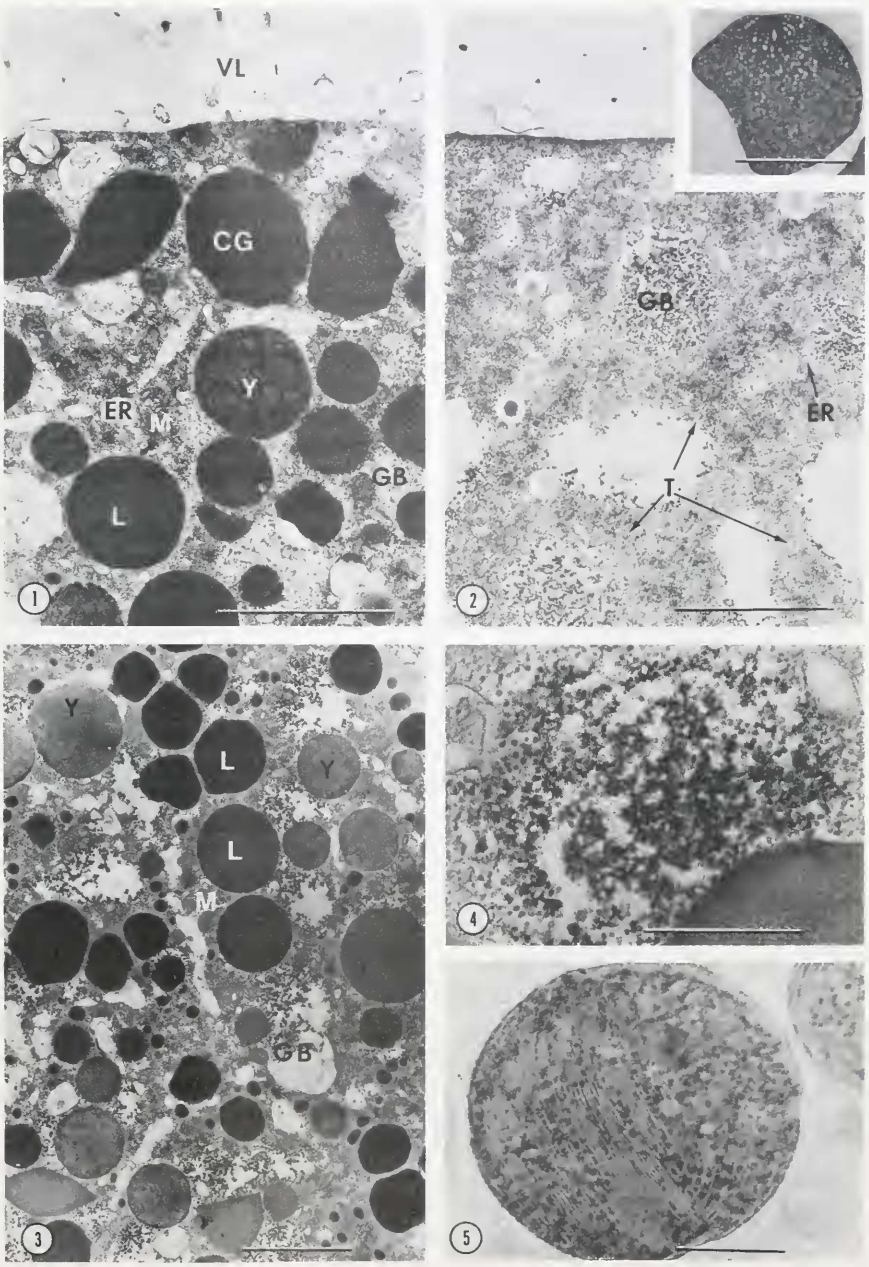
In the cleaving egg, the cortical region still possessed cortical granules. However, a 1 μm thick perivitelline space appeared between the vitelline layer and the plasma membrane (Fig. 6). Yolk, mitochondria, lipid, and smooth endoplasmic reticulum were distributed homogenously below the cortex. The spongy hyaloplasm became enclosed and contained occasional yolk platelets and lipid droplets, as well as numerous mitochondria in a matrix of microtubules and smooth endoplasmic reticulum (Fig. 7). The polar lobe showed no special structures. It contained the same cytoplasmic constituents as the surrounding cytoplasm, namely yolk, mitochondria, and lipid, which appeared to be arranged in rays running into the lobe (Fig. 8). The cortex of the polar lobe also appeared similar to that of the surrounding regions (Fig. 9). Granular bodies were interspersed with the cortical granules (Fig. 9). Cortical granules were reduced in number, but not completely absent, in cleavage constrictions. (Fig. 8).

Sixteen-cell embryo

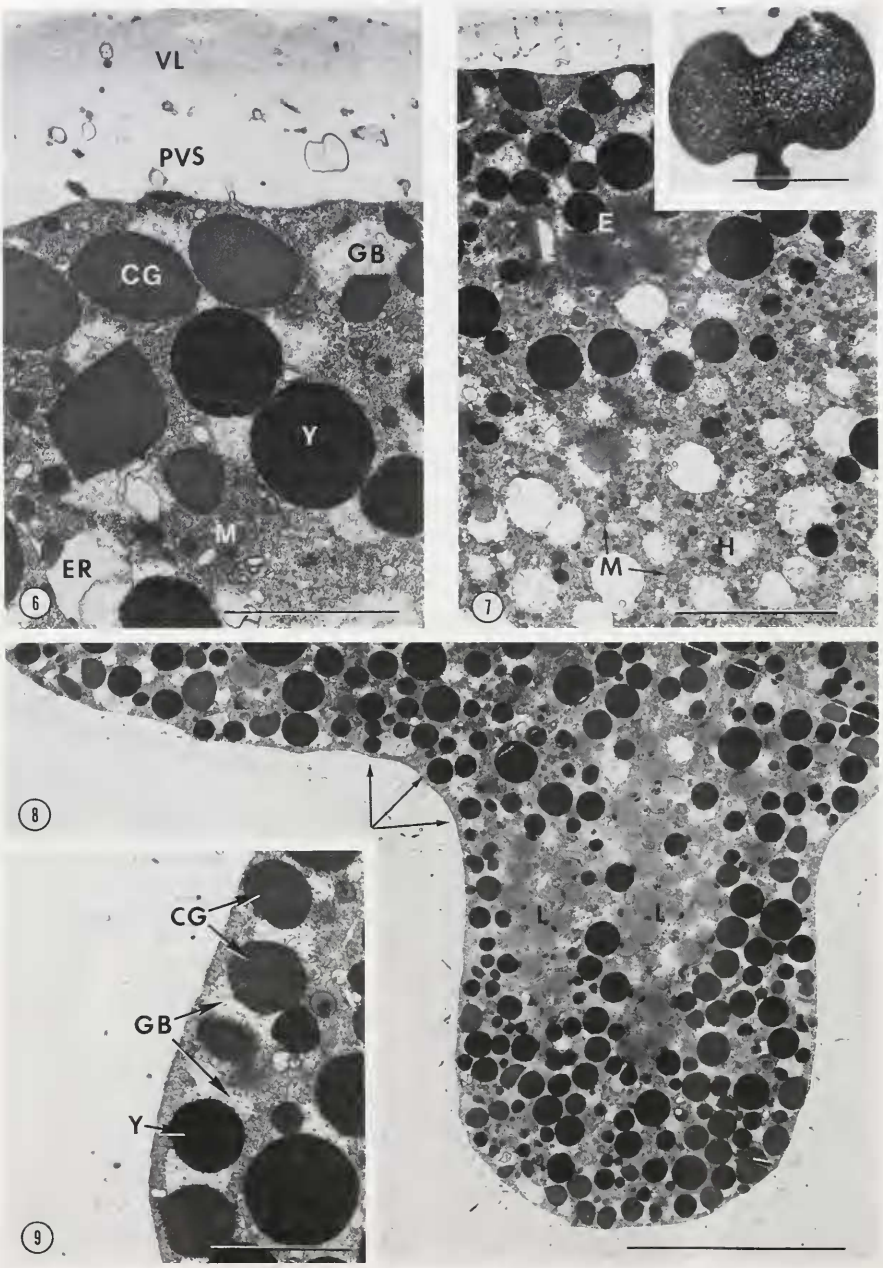
Figure 10 shows a blastomere of a 16-cell embryo. The cell was clearly polarized. The nucleus was eccentric, lying close to the cortex, which retained its cortical granules. The perinuclear hyaloplasm was spongy and possessed a network of microtubules and smooth endoplasmic reticulum (Fig. 11). Yolk and lipid were localized towards the segmentation cavity (Figs. 10, 12). Desmosomes anchored adjacent blastomeres to one another (Fig. 10, inset). Pores perforated the nuclear envelope.

Trochophore larva

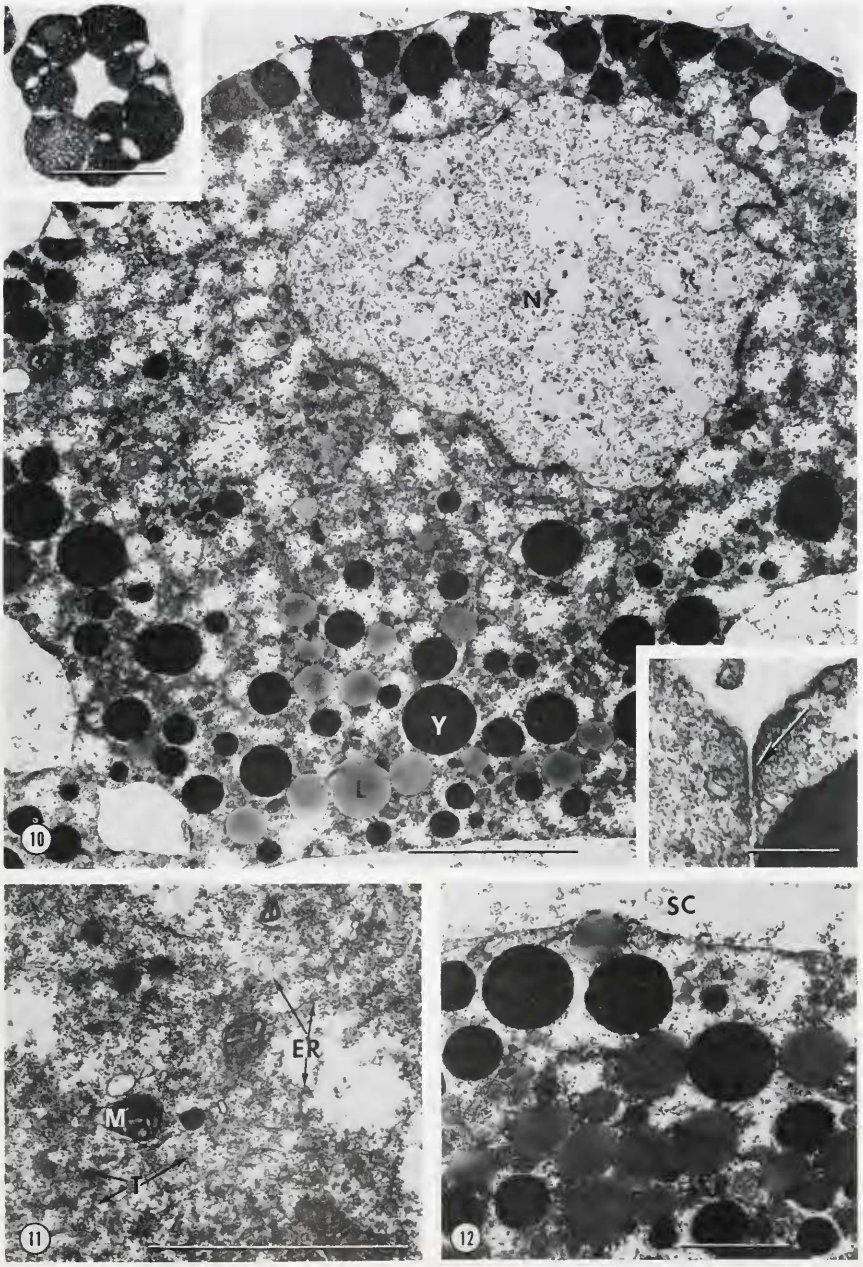
Embryos were followed to the early trochophore larval stage when the metatrochal band and apical organ were present, but the muscle bands had not fully



FIGURES 1–5. Figure 1. Section through the cortex of an unfertilized egg. Note cortical granules (CG), vitelline layer (VL), granular body (GB), mitochondrion (M), endoplasmic reticulum (ER), lipid (L), and yolk (Y). Scale bar = 2.0 μm. Figure 2. Section through the cortex of an unfertilized egg in the region of the spindle. Note granular body (GB), endoplasmic reticulum (ER), and microtubules (T), and the absence of other organelles. Scale bar = 2.0 μm. Inset: light micrograph of a thick section of an unfertilized egg, with meiotic spindle at the top. Note how hyaloplasm is arrayed by the spindle. Scale bar = 100 μm. Figure 3. Section through the endoplasm of an unfertilized egg showing granular bodies (GB), lipid (L), mitochondria (M), and yolk (Y). Scale bar = 2.0 μm. Figure 4. High magnification electron micrograph showing the fine structure of a granular body. Scale bar = 0.5 μm. Figure 5. High magnification electron micrograph showing the fine structure of a yolk granule. Scale bar = 0.5 μm.



FIGURES 6–9. Figure 6. Section through the cortex of a cleaving egg showing vitelline layer (VL), perivitelline space (PVS), cortical granules (CG), granular bodies (GB), endoplasmic reticulum, (ER), mitochondria (M) and yolk (Y). Scale bar = 2.0 μm. Figure 7. Low power electron micrograph of a section through a cleaving egg. Note the subcortical localization of the yolk and lipid of the endoplasm (E) and deeper localization of the hyaloplasm (H). Also note the presence of mitochondria (M) in the hyaloplasm. Scale bar = 5.0 μm. Inset: light micrograph of a cleaving egg. Scale bar = 100 μm. Figure 8. Low power electron micrograph through the polar lobe of a cleaving egg. Note rays of lipid (L) and similarity of polar lobe cytoplasm to surrounding endoplasm. Also note relative absence of cortical granules at furrows (arrows). Scale bar = 10 μm. Figure 9. Higher magnification electron micrograph through the cortex of the polar lobe showing association of granular bodies (GB) with cortical granules (CG) and yolk (Y). Scale bar = 2.0 μm.



FIGURES 10–12. Figure 10. Electron micrograph of a section through a blastomere of a 16-cell embryo. Note polarization of the cell with apical nucleus (N) and basal endoplasm with lipid (L) and yolk (Y). Scale bar = 5.0 μm. Upper inset: light micrograph of a section through a 16-cell embryo. Scale bar = 100 μm. Lower inset: electron micrograph showing a desmosome (arrow) at the junction between adjacent blastomeres. Scale bar = 1.0 μm. Figure 11. Section through the hyaloplasm of a 16-cell embryo. Note small traebeculae of endoplasmic reticulum (ER) and microtubules (T) and mitochondria (M). Scale bar = 2.0 μm. Figure 12. Section showing concentration of endoplasm near the segmentation cavity (SC). Scale bar = 2.0 μm.

differentiated and the archenteron lacked a well-defined lumen. Although the embryos were actively swimming, there was no evidence of "hatching" from the vitelline envelope. Instead, active cilia penetrated the envelope (Figs. 13, 14).

Cells near the apical end were columnar, with the nuclei away from the surface (Fig. 13). The cells had cilia and prominent cortical granules, mitochondria, and granular bodies.

Ectodermal cells of the metatroch (Fig. 14) had cilia with typical basal bodies, many mitochondria, a well-developed Golgi apparatus, vesicles of endoplasmic reticulum, and granular bodies. Cells were irregularly shaped and highly interdigitated. Desmosomes were at cell junctions near the embryo surface. Nuclear envelopes contained dense arrays of pores. Numerous microvilli lay among the cilia.

Figure 15 shows the postrochal region. Its cells had prominent microvilli but very few cilia. Endodermal cells containing yolk and lipid protruded between the ectodermal cells. All postrochal cells were irregularly shaped and very spongy, with abundant mitochondria and smooth endoplasmic reticulum.

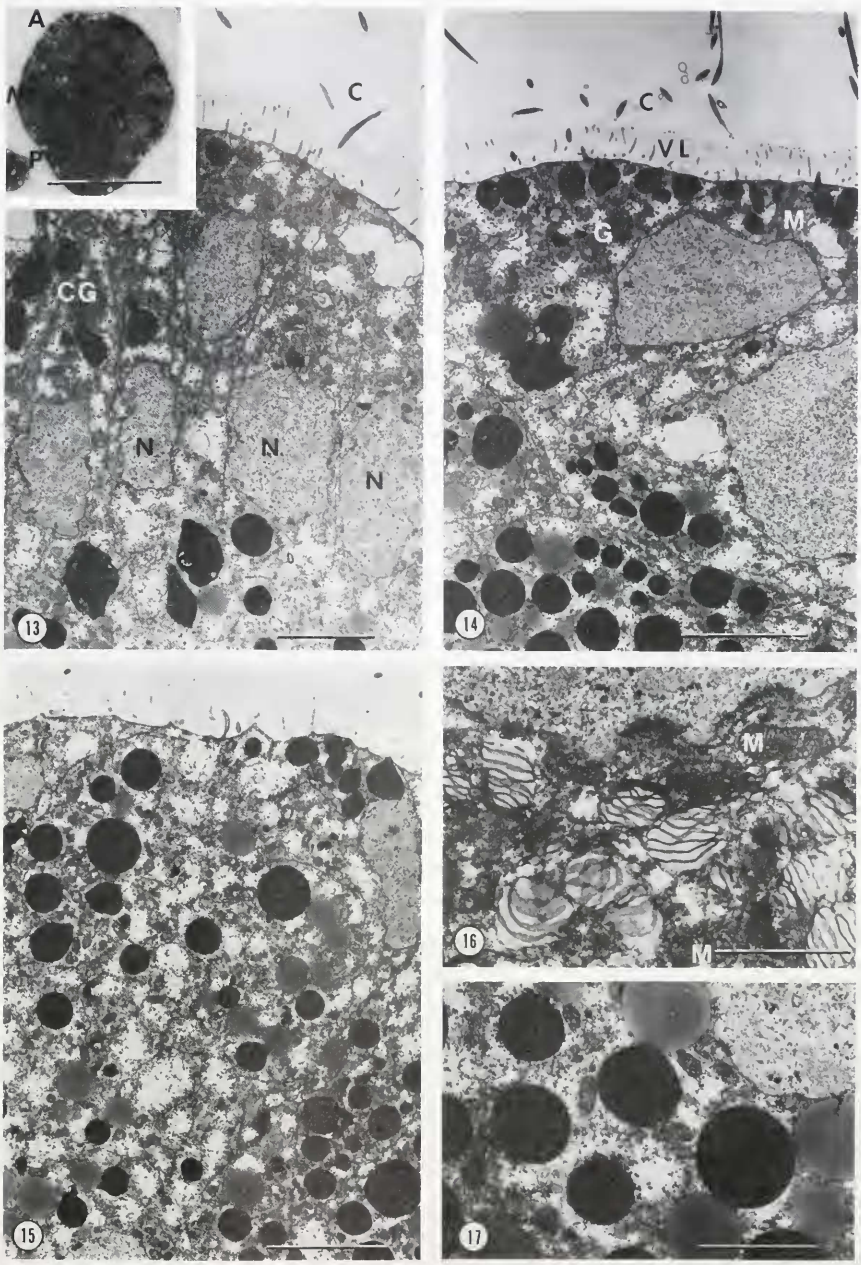
Mesodermal cells had numerous mitochondria, dilated vesicles of endoplasmic reticulum, and Golgi-like structures composed of stacks of membranes (Fig. 16).

Endodermal cells contained dense accumulations of yolk and lipid with associated mitochondria (Fig. 17) and fairly regularly-shaped nuclei with prominent nucleoli.

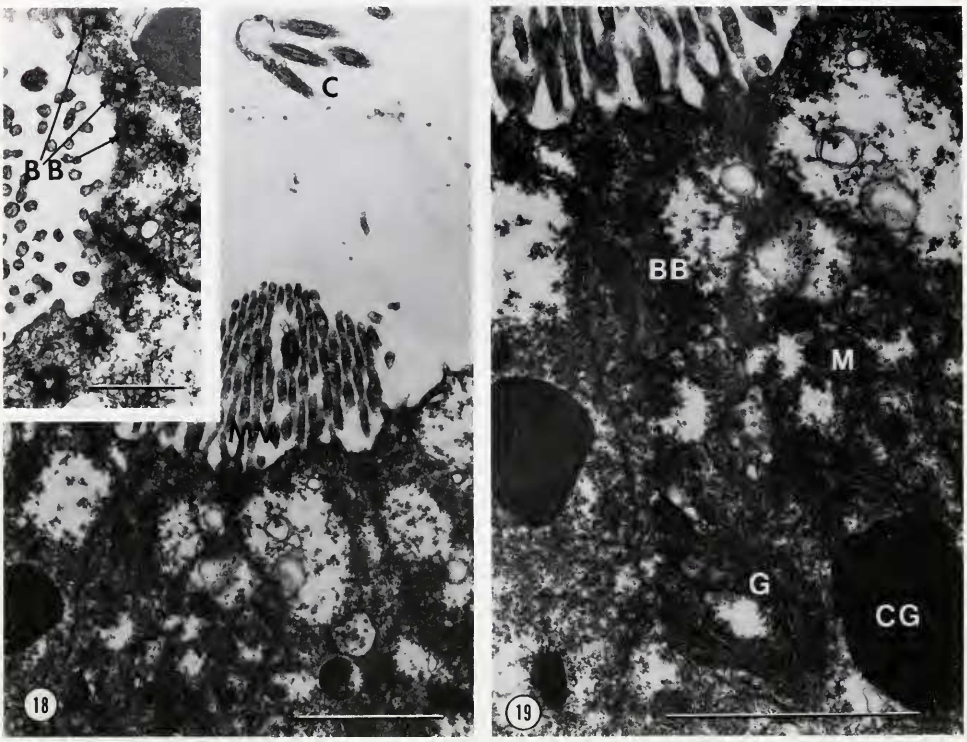
The apical organ was composed of several cilia surrounded by numerous microvilli up to 2 μm long, protruding from a group of cells forming a pit at the apical end (Fig. 13, inset; Fig. 18). Several cilia protruded from a single cell (Fig. 18, inset). Granular bodies were just below the surface, giving the apical organ a vesicular appearance (Figs. 18, 19). Mitochondria, Golgi apparatus, and vesicular endoplasmic reticulum were prominent. Cortical granules were present but deeper within the cells (Fig. 19).

Discussion

In an elegant series of studies, Lillie (1906, 1909) demonstrated the asymmetric localization of cytoplasmic components in *Chaetopterus* eggs and embryos, and the characteristic rearrangements of such components, which sequester them into different blastomeres. Based on staining properties, Lillie (1906) recognized (1) an ectoplasm, shown in the present study to be composed of cortical granules with interspersed granular bodies and vesicles of endoplasmic reticulum; (2) two granular endoplasms, shown here to be composed of yolk and lipid with interspersed mitochondria, granular bodies, and endoplasmic reticulum, and (3) a granule-free endoplasm, shown here to contain granular bodies in a matrix of microtubules and endoplasmic reticulum. For clarity, I call the granule-free endoplasm the hyaloplasm (*cf.* Wilson, 1928). Lillie (1909) recognized the morphogenetic importance of this material and noted that it is derived from the "residual substance" of the germinal vesicle. Its strong basophilia (Lillie, 1909) indicates the presence of acidic macromolecules. The present investigation showed the granular bodies to be composed of 18–20 nm structures aggregated into chains and larger granules. These small granules are approximately the size of ribosomes. The granular bodies are similar in structure to the polar granules of *Drosophila* (Mahowald, 1962) and the germinal granules of amphibians (*e.g.*, Williams and Smith, 1971). Which components of the granules of insects and amphibians carry morphogenetic significance is not known. These latter granules are associated with germinal differentiation, whereas the granular bodies in *Chaetopterus* were found in all tissues of the trochophore and in all parts of the early embryo.



FIGURES 13–17. Figure 13. Electron micrograph showing the apical region of a trochophore larva. Note columnar shape of cells, cilia (C), cortical granules (CG), and nuclei (N). Also note absence of yolk and lipid from ectoderm. Scale bar = 5.0 μm. Inset: light micrograph of a section through a trochophore larva to show locations of sections shown in Figures 13–15. A = apical region; M = metatrochal band; P = postrochal region. Scale bar = 100 μm. Figure 14. Electron micrograph of a section through the metatrochal band. Note cilia (C) penetrating the vitelline layer (VL). Also note Golgi apparatus (G) and mitochondria (M). Scale bar = 5.0 μm. Figure 15. Electron micrograph of a section showing the postrochal region. Note that endodermal cells containing yolk and lipid approach the surface but are still surrounded by ectodermal cells. Also note relative lack of cilia. Scale bar = 5.0 μm. Figure 16. Section through a mesodermal cell. Note dilated stacks of membranes and numerous mitochondria (M). Scale bar = 2.0 μm. Figure 17. Typical section through an endodermal cell. Scale bar = 2.0 μm.



FIGURES 18–19. Figure 18. Section through the apical organ showing microvilli (MV) and cilia (C). Inset: three basal bodies may be seen in one cell of the apical organ (BB, arrows). Scale bar = 1.0 μm. Figure 19. Higher magnification electron micrograph of apical organ cells showing basal bodies (BB), cortical granules (CG), and Golgi apparatus (G). Scale bar = 2.0 μm.

In the unfertilized egg, the hyaloplasm was in the spindle region and excluded structures such as mitochondria, yolk, lipid, and cortical granules (ectoplasmic defect of Lillie, 1906). After fertilization, mitochondria moved into the area in abundance, although it still contained little yolk or lipid.

The structure of the hyaloplasm persisted throughout cleavage and gastrulation, although its position in the embryo changed. In accordance with previous reports (Lillie, 1902, 1906, 1909), the hyaloplasm was at the interior of the cleaving egg and eventually moved to more superficial regions. In the trochophore, the hyaloplasm was mostly in the ectoderm.

The only change in the egg cortex upon fertilization was the appearance of a perivitelline space. This was not due to cortical granule breakdown, because the cortical granules persisted to the trochophore. Humphreys (1967) reported a similar situation in *Mytilus*. The endoplasm was not distinct from the ectoplasm during early development.

At first cleavage, the endoplasm was peripheral and completely enclosed the hyaloplasm. Endoplasm filled the polar lobe. This contradicts Lillie's (1906) report that the polar lobe is primarily ectoplasmic. Whitaker and Morgan (1930) also

found the polar lobe to be endoplasmic. Mead (1895) reported astral rays penetrating the polar lobe. The lipid aggregates shown in this report may have been those rays.

With the exception of Dohmen and Verdonk (1979), using *Bithynia*, no specialized structures of potential morphogenetic importance have been detected in studies of molluscan polar lobes (Reverberi and Mancuso, 1961; Crowell, 1964; Humphreys, 1964; Reverberi, 1970) although some structures have been reported to be concentrated in polar lobes (*e.g.*, mitochondria, Reverberi, 1970; double-membrane vesicles, Crowell, 1964). Since *Bithynia* has a small polar lobe, Dohmen and Verdonk (1979) suggested that small polar lobes might generally contain special structures. The annelid, *Chaetopterus*, also has a small polar lobe, but the present study showed no lobe-specific structures.

By the 16-cell stage, the endoplasm was oriented toward the basal end of all cells, as reported by Lillie (1906). This arrangement is maintained through cleavage and may be responsible for sequestering the endoplasm into the presumptive endodermal cells into which it ultimately passes. In the trochophore larva, endoplasmic structures were lacking in ectodermal cells, while cortical granules were lacking in endodermal cells as reported by Lillie (1906). This is consistent with the polarization of cells in the 16-cell embryo, although the endodermal cell lineage is not completely segregated at that stage (Mead, 1897).

Lillie (1906) reported that the apical organ consists of a flagellum protruding from cells lacking ectoplasm. The present report showed that the flagellum was a bundle of cilia and that the cells of the apical organ did contain cortical granules, although the granules were not positioned superficially.

Certain features of cytoplasmic localization were striking in *Chaetopterus* development. Ooplasmic segregation resulted in larval cells with distinct and characteristic cellular inclusions. The mechanism underlying this localization is not yet known with certainty. Microtubules and endoplasmic reticulum were arranged in a network throughout the cytoplasm. Since this network appeared to surround at least some of the localized organelles, it may contribute to cytoplasmic localization. Lillie (1906, 1909) and Wilson (1928) stressed the importance of such "ground substances" in localization.

Centrifugal forces sufficient to stratify the large organelles did not result in abnormal development of *Chaetopterus* (Lillie, 1906, 1909; Wilson, 1929, 1930) or other spirallians (Morgan, 1933, 1935; Clement, 1968). Electron microscopic examinations of centrifuged spirallian eggs have not reported any persisting microtubule or endoplasmic reticulum network, but neither have these been previously reported in uncentrifuged eggs. Vesicles of endoplasmic reticulum are found throughout centrifuged *Mytilus* eggs, however (Humphreys, 1962). Clearly an analysis of centrifuged *Chaetopterus* eggs by electron microscopy is warranted.

It has been proposed that morphogenetic information is localized in the cortex of the egg (e.g., Wilson, 1928; Davidson, 1976), but cellular structures that might localize morphogenetic determinants have not been detected. If such information is localized in the form of informational RNA molecules, as has often been supposed (see Davidson, 1976, for review), granular bodies, if they are shown to contain informational RNA, would be prime candidates for such localization. Because of their organization within the egg, they are probably sequestered along with other cytoplasmic components in *Chaetopterus* embryos.

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